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THE PRIMARY TOXICITY OF CERTAIN PREPARATIONS FROM TUBERCLE BACILLI FOR MICE AND GUINEA-PIGS

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Experiments to isolate the specific immunizing or toxic substances from the tubercle bacillus have been countless. Books have been written on this subject, and still we have no concise information as to whether the tubercle bacillus produces toxic substances or whether on disintegration a toxic element is produced that acts on the animal body. Vaughan¹ believes that he has prepared the true toxic substance not only from the tubercle bacillus, but also from many bacteria and pure protein substances by disintegrating them with alcoholic sodium or potassium hydroxid. That the tubercle bacillus does not produce a true bacterial toxin, as is the case with the diphtheria and tetanus bacilli, cannot be questioned but whether it produces a substance possessing immunizing or true poisonous properties is still debatable. In 1918, Corper and Sweany² reported observations on tubercle bacilli indicating that these organisms, both human and bovine, possessed autolytic and a number of other hydrolytic enzymes. In the surrounding autolysate there was found to be a certain correlation between the autolysis and antigen formation. Believing that there might exist, as with the pneumococcus, a certain relation between autolysis and the formation of a toxic substance in some way related to virulence, it was decided to prepare autolysates from virulent and avirulent, human and bovine tubercle bacilli and compare their toxicities to mice, and as far as possible to guinea-pigs. Likewise it was thought that a lysis of the bacilli by means of distilled water might liberate substances which though toxic may disappear on autolysis. The later conception was strengthened by the observations by Roger,³ in which rabbit blood laked by distilled water was toxic to rabbits, but this toxicity was absent or markedly less in autolyzed blood. It seemed desirable, therefore, to prepare distilled water extracts of

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¹ Poisonous Proteins, 1917.

² Jour. Bacteriol., 1918, 3, p. 129.

³ Arch. de méd. exper. et d'anat. path., 1918, 28, p. 325.

virulent and avirulent, human and bovine tubercle bacilli, and compare these with each other and with the autolysates from the same bacilli. In the preparation of the autolysates and distilled water extracts, the tubercle bacilli⁴ were obtained from glycerol broth cultures about 7 to 9 weeks old. The cultures were washed with 3 to 4 changes of sterile 0.9% sodium chloride P_H 7, Sørensen's standards, and transferred to sterile 15 c c graduated centrifuge tubes, 4 c c of bacilli in each tube, to which were then added either 10 c c of sterile 0.9% sodium chloride P_H 7 for those intended for the preparation of the autolysate, or 10 c c of sterile distilled water (P_H 7) for the preparation of the watery lysate. The autolysate was prepared by placing the bacilli, in the salt solution, in the incubator at 37 C. and keeping them there, shaking them daily, for 2 weeks when the supernatant clear fluid was obtained by high speed centrifugation. This was used for toxicity tests in mice and guinea-pigs, being preserved for use on ice. The fluids were all tested for sterility before injection. The watery lysate was prepared by placing the bacilli, in the distilled water, in the incubator at 37 C. for about 12 to 24 hours when they were placed in the icebox for the remainder of the 2 weeks, being shaken every few days. The supernatant extract was obtained by centrifugation, made isotonic by the addition of sterile sodium chloride, and was kept on ice, after testing its sterility, throughout the experiment.

The culture of avirulent human tubercle bacilli was one that was isolated in 1908 and proved avirulent for guinea-pigs, producing no demonstrable disease in doses of 1 mg. The culture of virulent human tubercle bacilli (Maxfield) was isolated from the sputum by Petroff's method in 1919 and produced generalized tuberculosis in guinea-pigs in a dose of 0.000,000,001 mg. administered subcutaneously. The culture of avirulent bovine tubercle bacilli was isolated in 1910 and produced no demonstrable disease in guinea-pigs in amount of 1 mg. given subcutaneously. The culture of virulent bovine tubercle bacilli was isolated in 1919 and produced generalized disease in the guinea-pig in a dose of 0.000,000,1 mg. subcutaneously. Each preparation was tested by a single injection of 2 c c intraperitoneally into one mouse, and by daily injections of 1 c c intraperitoneally into 2 other mice, as stated in the table. The animals used were young mice about 4 to 5 months old.

⁴ All of these tubercle bacilli were true tubercle bacilli, requiring at least 3 to 6 weeks to obtain a good growth as distinguished from certain rapidly, 2 to 4 day, growing acid fast micro-organisms which have been used in many of the investigations on this organism.

The mice were killed by means of ether and were carefully examined for macroscopic changes.

These experiments indicate that neither the autolysate nor water lysate of either human or bovine tubercle bacilli have an appreciable toxicity toward mice when given intraperitoneally in about 1 c c daily for 7 to 9 days.

TABLE 1
THE TOXICITY OF CERTAIN TUBERCLE BACILLUS PREPARATIONS, THE AUTOLYSATE AND DISTILLED WATER-LYSATE, FOR MICE

Preparation	Number of Mice Used	Intraperitoneal Injections Given in C c	Results
Human avirulent autolysate....	1	2 single.....	Negative
	2	1 daily for 9 days.....	Negative
Human avirulent water-lysate..	1	2 single.....	Negative
	2	1 daily for 4 and 10 days..	1 died from other causes
Human virulent autolysate.....	1	2 single.....	Negative
	2	1 daily for 8 and 9 days...	Negative
Human virulent water-lysate....	1	2 single.....	Negative
	2	1 daily for 8 and 9 days...	Negative
Bovine avirulent autolysate.....	1	2 single.....	Negative
	2	1 daily for 7 and 8 days...	Negative
Bovine avirulent water-lysate...	1	2 single.....	Negative
	2	1 daily for 7 days.....	Negative
Bovine virulent autolysate.....	1	2 single.....	Negative
	2	1 daily for 4 and 8 days...	1 died from other causes
Bovine virulent water-lysate....	1	2 single.....	Negative
	2	1 daily for 7 and 8 days...	Negative

TABLE 2
THE TOXICITY OF CERTAIN TUBERCLE BACILLUS PREPARATIONS, THE AUTOLYSATE AND WATER-LYSATE FOR GUINEA-PIGS

Preparation	Number of Guinea-Pigs Used	Intraperitoneal Injection in C c	Results
Human avirulent autolysate....	2	10	Slight immediate reaction due to the amount of fluid
Human avirulent water-lysate..	1	10	Slight immediate reaction due to the amount of fluid
Human virulent autolysate.....	1	8	No reaction
Human virulent water-lysate....	1	10	No appreciable reaction
Bovine avirulent autolysate.....	1	9	No appreciable reaction
Bovine avirulent water-lysate...	2	10	Slight immediate reaction due to the amount of fluid
Bovine virulent autolysate.....	1	8	No reaction
Bovine virulent water-lysate....	1	8	No appreciable reaction

Besides these tests in mice the preparations of the human and bovine tubercle bacilli were tested, so far as feasible with the material at hand, in guinea-pigs. Unfortunately, only sufficient material was available for a single large injection. Young guinea-pigs were used weighing about 300 gm. each. The results on the guinea-pigs are given in table 2.

The experiments on the guinea-pigs indicate that amounts of from 8 to 10 c c of the autolysate or water-lysate, of virulent or avirulent, human or bovine tubercle bacilli, injected intraperitoneally have no appreciable toxic action on these animals. When we consider these experiments in the light of observations by Wherry and Ervin,⁵ who found that the mere grinding of lung tissue from the rabbit with 0.9% sodium chloride (0.6 gm. of lung tissue ground with 6 c c of solution) produces an extract sufficiently toxic to kill a 2 kilo rabbit when given intravenously in 0.3 c c amounts, comparatively at least, the tubercle bacillus extracts seem to be quite innocuous to animals.

Likewise 0.5 c c of an extract of the lungs of guinea-pigs given intravenously was found to be fatal to guinea-pigs. Similar extracts of liver, kidney, ileum and spleen were found relatively nontoxic to rabbits by Wherry and Ervin,

SUMMARY

The autolysate or water-lysate prepared from tubercle bacilli avirulent and virulent human and avirulent and virulent bovine bacilli in proportion of 4 parts by volume of bacilli to 10 of fluid possess no toxicity for mice when injected intraperitoneally in a single dose of 2 c c or daily for 9 days in a dose of 1 c c, nor for guinea-pigs in a single intraperitoneal injection as large as 10 c c.

⁵ Jour. Infect. Dis., 1918, 23, p. 240.